### Cytoscape Model SEED Plugin Tutorial

#### **GOAL OF THE CYTOSEED PLUGIN**

The goal the CytoSEED plugin is to enable users of the Model SEED (<a href="http://www.theseed.org/models">http://www.theseed.org/models</a>) to view, manipulate and analyze metabolic models created by the Model SEED. At this point CytoSEED does not enable users to edit their models; any modifications made to the model visualization in CytoSEED will not be reflected on the Model SEED website (though this is a capability we intend to add in future releases).

CytoSEED takes a reaction-centric view of metabolic models. When a model is loaded into CytoSEED, the reactions are organized into a set of maps based on each reaction's membership in KEGG pathways. A map is created for each KEGG pathway represented in the model, and some reactions may appear in multiple maps. Compounds are moved, hidden, and shown with their respective reactions. CytoSEED is particularly useful for trimming superfluous maps from the model's representation and organizing the remaining maps into an informative visualization of the results of flux-balance analysis.

CytoSEED is also useful for comparing multiple metabolic models, whether multiple models of the same organism, or models of different organisms. See details for loading multiple models below.

CytoSEED is released under the GNU General Public License. Please contact Matt DeJongh at Hope College (dejongh@hope.edu) with questions, problems and suggestions.

### INSTALLING THE CYTOSEED PLUGIN

- 1. The CytoSEED plugin is compatible with all Cytoscape 2.8 releases. Download and install the latest Cytoscape 2.8 release from http://cytoscape.org.
- 2. Download the CytoSEED plugin from <a href="http://sourceforge.net/projects/cytoseed/">http://sourceforge.net/projects/cytoseed/</a>. Start the Cytoscape application and install the CytoSEED plugin by clicking on the *Plugins -> Install Plugin from File* menu item; then browse to and select the *CytoSEEDplugin.jar* file that you downloaded.
- 3. In order to format the KEGG maps properly, you must also install the *KGMLReader* plugin, which is available from within the Cytoscape application. Click on the *Plugins -> Manage Plugins* menu item; in the box labeled "Enter key words to search", type "kgml", then click on the "Search" button. Click on the arrow beside the "Network and Attribute I/O" folder, select the latest version of the KGMLReader plugin, and click on "Install".
- 4. If you installed both plugins correctly, you will now see new menu items listed under the Cytoscape *Plugins* menu as *KGML Reader* and *SEED*. Select the *Plugins -> SEED -> Set Location of CytoSEED Folder* menu item, and choose a location where the plugin can create a new folder named *CytoSEED* that will store the model and KGML data (you do not need to create this folder yourself, the plugin will create it for you). Next you will be prompted to download the KGML files from KEGG; click on "Yes". This will take a few minutes. If there are problems with the download, you can restart it at any time from the *Plugins -> SEED -> Download KGML Files From KEGG* menu item.

## LOADING A MODEL USING THE CYTOSEED PLUGIN

- 1. Click on Plugins -> SEED -> Load Models And Create Session (Ctrl+Shift+L)
- 2. Enter Model IDs by typing in the text box (if loading private model(s), also enter RAST account username and password), then click OK. The following examples use the M. genitalium G-37 model (Seed243273.1).

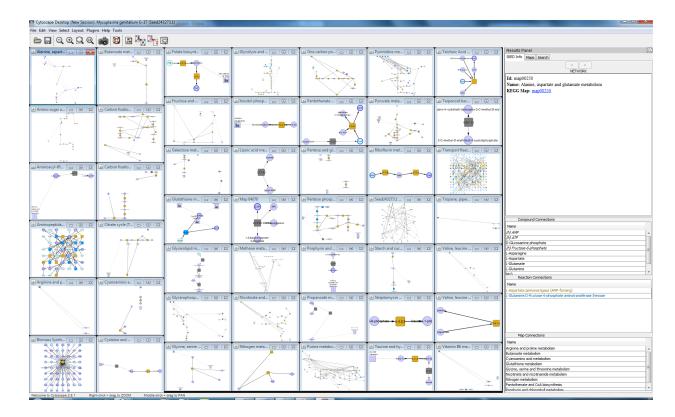
Note: The process of downloading the model data from the Model SEED server takes a few minutes; the data is cached in the local *CytoSEED* folder created during the installation process, so subsequent loading of the model data is much faster. If at any time you wish to reload the model data from the Model SEED server (for example, if the model has been updated on the server), check the "Choose models to reload from server" option in the Load Models And Create Session dialog.



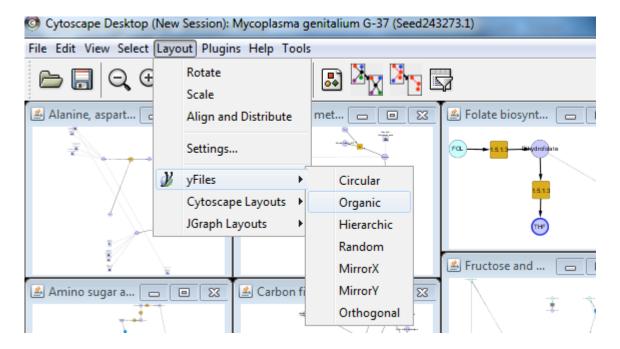
CytoSEED will lay out most KEGG maps automatically if the KGMLReader Plugin has been installed as described above and you are connected to the internet (KGML validation requires an internet connection). CytoSEED also creates maps for Transport Reactions, Biomass Reactions, and any leftover reactions that aren't assigned to KEGG maps. The leftover reactions go in the map named Seed243273.1 Network.

Each map only displays those compounds that are present in the corresponding KEGG map; any other compounds associated with reactions in the map are hidden. In addition, common compounds such as water and ATP are hidden by default. In a few cases, this means that all of a reaction's compounds are hidden by default (e.g., ATP synthase); any hidden compound can be unhidden if desired (see below)

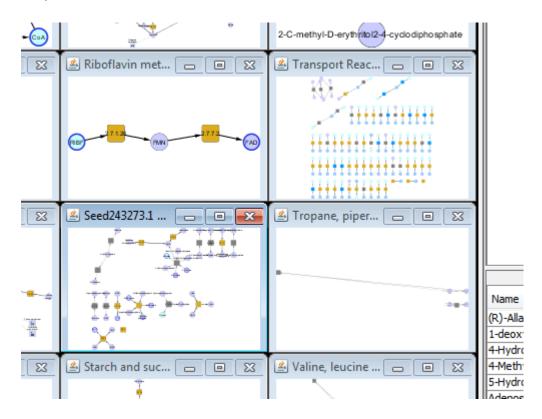
When the model data is loaded and the maps have been formatted, CytoSEED will display a tiled view of all the maps, arranged in alphabetical order by columns. If you modify the size of the Cytoscape window, you will need to re-tile the maps by selecting the menu item *Plugins -> SEED -> Tile All Maps* (Ctrl-Shift-T).



When the model is done loading, select the Seed243273.1 Network by clicking on its title bar. Click on Menu: Layout -> yFiles -> Organic or Circular to give it a nice layout.



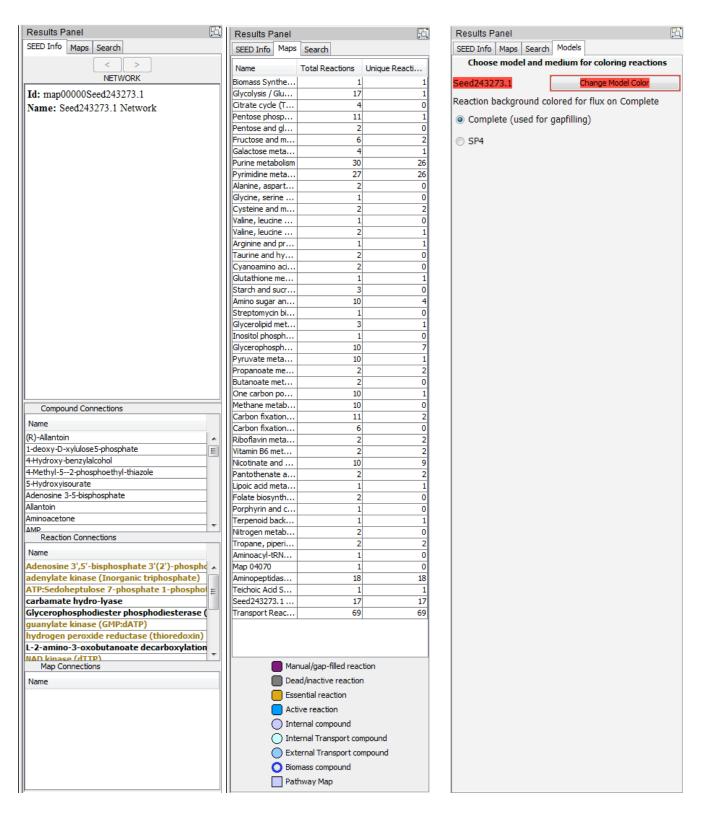
Repeat with Transport Reactions Map. Because of the large number of reactions and compounds present in the Transport Reactions Map, the names of reactions and compounds are not shown by default. To see them, zoom in on the map, or select the *View->Show Graphics Details* menu item.



Note: Every now and then Cytoscape gets confused about where map windows are supposed to be displayed as they are maximized and minimized. It is best to maximize and minimize map windows by hand, using the controls on each map window's title bar. Once you have minimized all the map windows, you can use Ctrl-Shift-T to rearrange them in alphabetical order and zoom them to full view.

#### **RESULTS PANEL**

The right side Results Panel has 4 tabs - *SEED Info* (shows information about selected map/node(s) and provides buttons at the top to allow for scrolling through previously selected nodes (keyboard shortcuts: Ctrl-Shift-J / Ctrl-Shift-K); *Maps* (shows information about Total Reactions and Unique Reactions in each map); *Search* (by id, EC number, or name); and *Models*, which shows which model(s) are loaded, and the media for which flux variability analysis results are available (radio buttons for each medium enable switching the coloration of reactions for FVA results on the selected medium – see below).



The Models panel on the right shows that there are flux variability analysis results for the Seed243273.1 model on both Complete and SP4 media.

### **UNDERSTANDING COLORATION OF CYTOSEED MAPS**

Reactions are colored based on their possible flux values given the currently selected medium; the currently selected medium is displayed in the Cytoscape Desktop window title bar, and can be changed using the *Plugins->SEED->Choose Media for Flux Display* menu item (Ctrl-Shift-M)

- Essential Must have flux for biomass growth on selected medium
- Active Can have flux, but not necessary for biomass growth on selected medium
- Inactive No flux possible through reaction on selected medium
- Gap-filled Reaction added by gapfilling process; in this case a color band across the top of the reaction will display whether it is Essential, Active or Inactive

# Compounds

- Intracellular compound
- Intracelluar compound that is the substrate or product of a transport reaction
- Extracelluar compound that is the substrate or product of a transport reaction

#### **Network Node**

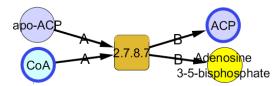
Network Node - Denotes connection between maps

Bold arrows denote reactions that can carry flux.

Reaction Flowing Bidirectionally (arrows leave reaction in both directions)



Right click on the reaction and select *Show Substrate/Product Labels* to distinguish substrates (labeled *A*) from products (labeled *B*)



Reaction Flowing in one direction



Compound Present in Another Map



# MANIPULATING THE CYTOSEED VIEW OF THE MODEL

1. Select the Maps tab within the Results panel. The widths of the Name, Total Reactions, and Unique Reactions columns can be adjusted using the mouse (hover over the bar between the column headers; click and pull the bar in the appropriate direction).

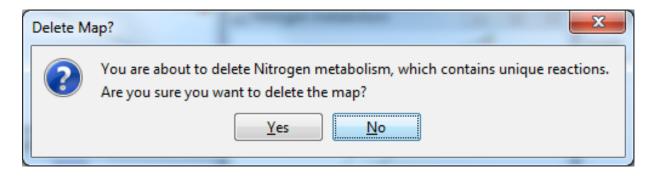
SEED Info Maps Search		
Name	Total Reactions	Unique Reactions
Biomass Synthesis Reactions	1	
Glycolysis / Gluconeogenesis	17	
Citrate cycle (TCA cycle)	4	
Pentose phosphate pathway	11	
Pentose and glucuronate interconversions	2	
Fructose and mannose metabolism	6	
Galactose metabolism	4	
Purine metabolism	30	2
Pyrimidine metabolism	27	2
Alanine, aspartate and glutamate metabolism	2	
Glycine, serine and threonine metabolism	1	
Cysteine and methionine metabolism	2	
Valine, leucine and isoleucine degradation	1	
Valine, leucine and isoleucine biosynthesis	2	
Arginine and proline metabolism	1	
Taurine and hypotaurine metabolism	2	
Cyanoamino acid metabolism	2	
Glutathione metabolism	1	
Starch and sucrose metabolism	3	
Amino sugar and nucleotide sugar metabolism	10	
Streptomycin biosynthesis	1	
Glycerolipid metabolism	3	
Inositol phosphate metabolism	1	
Glycerophospholipid metabolism	10	
Pyruvate metabolism	10	
Propanoate metabolism	2	
Butanoate metabolism	2	
One carbon pool by folate	10	
Methane metabolism	10	
Carbon fixation in photosynthetic organisms	11	
Carbon fixation pathways in prokaryotes	6	
Riboflavin metabolism	2	
Vitamin B6 metabolism	2	
Nicotinate and nicotinamide metabolism	10	
Pantothenate and CoA biosynthesis	2	
Lipoic acid metabolism	1	
Folate biosynthesis	2	
Porphyrin and chlorophyll metabolism	1	
Terpenoid backbone biosynthesis	1	
	2	
Nitrogen metabolism Tropane, piperidine and pyridine alkaloid biosynthesis	2	
	1	
Aminoacyl-tRNA biosynthesis Map 04070	1	
•		
Aminopeptidases (auto-generated)	18	
Teichoic Acid Synthesis (auto-generated)	1	
		1 6
Seed 243273. 1 Network Transport Reactions	17 69	
Manual/gap-filled re	action	
Dead/inactive reacti	on	
Essential reaction		
Active reaction		
Internal compound		
<ul> <li>Internal Transport of</li> </ul>	ompound	
External Transport	compound	
<u>~</u>	- Pour	
O Biomass compound		
External Transport of Biomass compound Pathway Map	•	

2a.Click the Unique Reactions column header to sort in ascending order. Shift-select those with zero unique reactions; these maps are good candidates for deleting from the CytoSEED view. Delete them by right-clicking on any of the selected maps and selecting Delete Everywhere

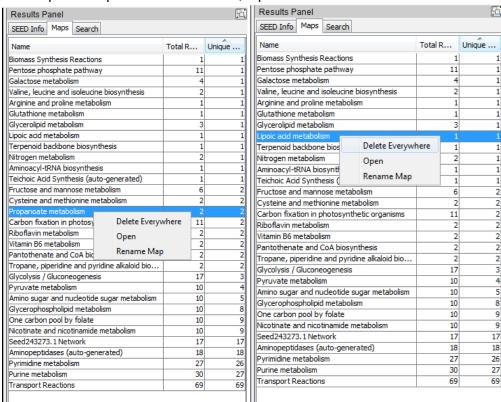
SEED Info Maps Search				
		Tatal Danation		Lleieus Passtian
Name		Total Reactions		Unique Reaction
Citrate cycle (TCA cycle)			4	
Pentose and glucuronate interconversion			2	
Alanine, aspartate and glutamate metab			2	
Glycine, serine and threonine metabolism				
/aline, leucine and isoleucine degradatio	n			
Faurine and hypotaurine metabolism				
Cyanoamino acid metabolism			2	
Starch and sucrose metabolism				
Streptomycin biosynthesis				
(nositol phosphate metabolism				
Butanoate metabolism			2	
Methane metabolism			10	
Carbon fixation pathways in prokaryotes				
Folate biosynthesis			2	
orphyrin and chlorophyll metabolism	Delete Ev	verywhere		
litrogen metabolism				
Aminoacyl-tRNA biosynthesis				
1ap 04070				
Biomass Synthesis Reactions			1	
Glycolysis / Gluconeogenesis			17	
Pentose phosphate pathway			11	
Galactose metabolism			4	
Valine, leucine and isoleucine biosynthesi	is		2	
Arginine and proline metabolism			1	
Glutathione metabolism			1	
Glycerolipid metabolism			3	
Pyruvate metabolism			10	
One carbon pool by folate			10	
ipoic acid metabolism			1	
Terpenoid backbone biosynthesis		1		
eichoic Acid Synthesis (auto-generated)	)		1	
ructose and mannose metabolism	•		6	
ysteine and methionine metabolism			2	
ropanoate metabolism			2	
Carbon fixation in photosynthetic organi	sms		11	
Riboflavin metabolism			2	
/itamin B6 metabolism		2		
Pantothenate and CoA biosynthesis			2	
ropane, piperidine and pyridine alkaloid	biosynthesis		2	
Amino sugar and nucleotide sugar metab			10	
Glycerophospholipid metabolism			10	
Vicotinate and nicotinamide metabolism			10	
Seed 243273, 1 Network			17	
Aminopeptidases (auto-generated)			18	
Purine metabolism			30	
Pyrimidine metabolism			27	
Fransport Reactions			69	
ransport Reactions			09	ı '

The maps are deleted one by one. Every time a map is deleted, the unique reactions for all maps are recalculated, so there may be maps selected for deletion that end up with unique

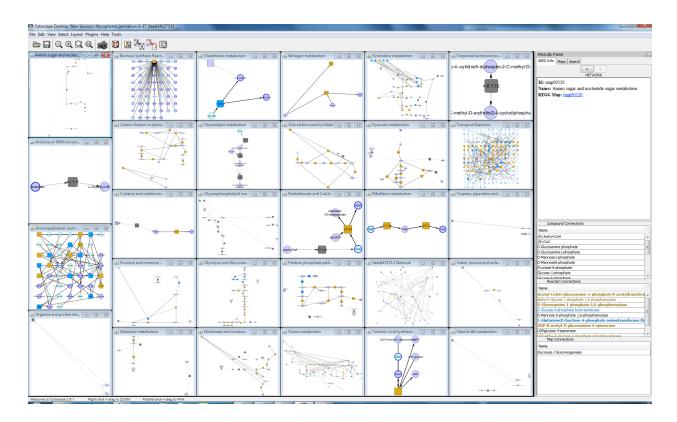
reactions once other maps are deleted. You will be prompted to decide if you would like to delete maps containing unique reactions:



- 4. You may use this same technique to delete any maps that you determine are not relevant for understanding the organism
- Examples: Propanoate Metabolism, Lipoic Acid Metabolism



The tiled view will automatically update after maps are deleted:



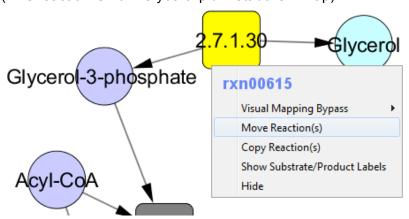
## **MAXIMIZING AND RETILING MAPS**

- 1. Click on the "maximize" control in the map window's title bar to maximize that map.
- 2. Ctrl-Shift-Z will zoom the map to fit the new window size.
- 3. Click on the "minimize" control in the map window's title bar to minimize that map.
- 3. Ctrl-Shift-T will restore the zoom in the tiled view of all the maps.

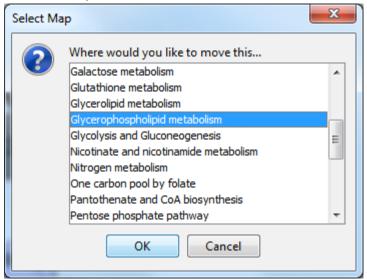
### **MOVING REACTIONS BETWEEN MAPS**

1. Right click on the reaction and select "Move Reaction(s)"

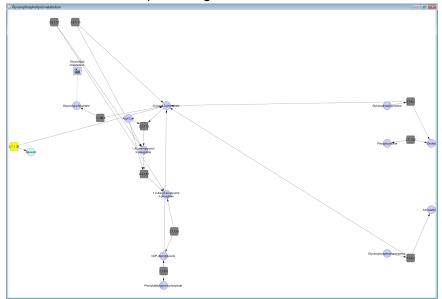
(This reaction is from Glycerolipid Metabolism map)



# 2. Select map destination



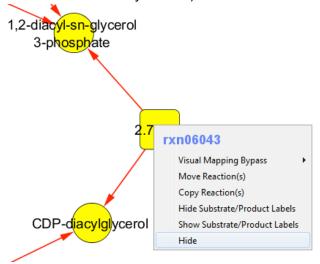
3. The destination map is brought forward with moved reaction and connected compounds.



The reaction and associated compounds can be moved by selecting them and dragging them

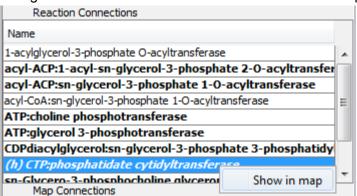
### HIDING REACTIONS AND COMPOUNDS

1. Select nodes to be hidden. To select multiple nodes, hold down the Shift key and click on each one, or left-click in the background and drag a box around the nodes. Then right-click on any of the selected nodes and select Hide (if a reaction is hidden, connected compounds may also be automatically hidden)



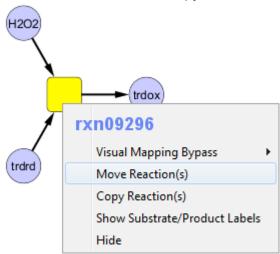
To Show a Hidden Node (designated by *italics* and "(h)" in the Results Panel)

1. Right click on Name in Connections area of Results panel and select Show in Map

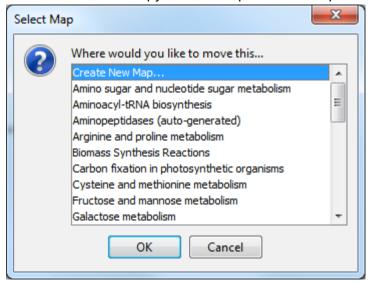


### **CREATING A NEW MAP**

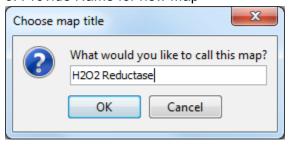
1. Shift-Select Reactions to be moved; then right-click on one of the selected reactions and select Move Reactions or Copy Reactions



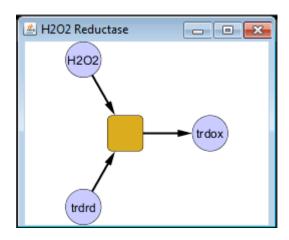
2. Within the move/copy reactions options is the option to create a new map



3. Provide Name for new Map

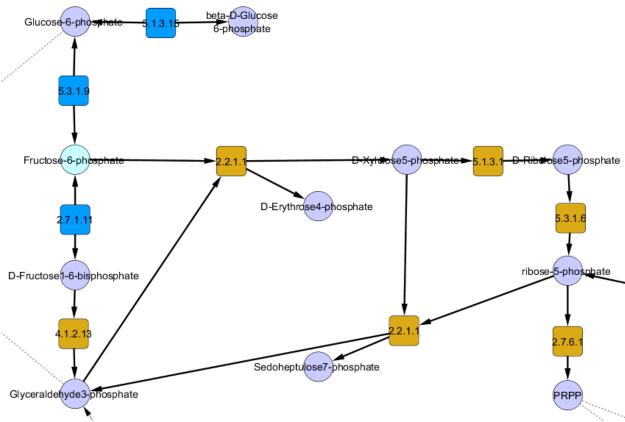


4. Newly created map. Use Ctrl-Shift-T to rearrange the maps in the tiled view.

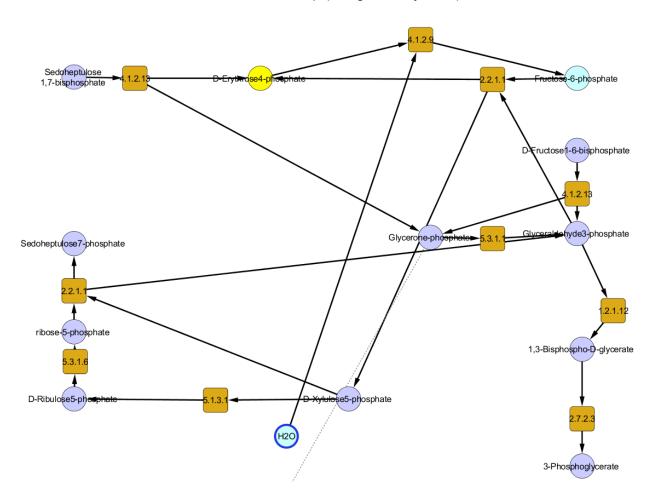


## **FILLING GAPS IN MAPS**

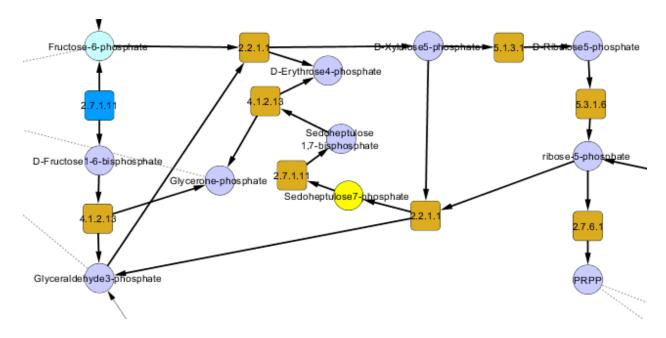
1. In the Pentose Phosphate Pathway, there is a gap between Erythrose 4-phosphate (E4P) and Sedoheptulose 7-phosphate (S7P). However, the bold arrows show that there is flux through these compounds. Where are the reactions?



2. Select just E4P. The "Map Connections" panel shows that the compound is present in the "Carbon fixation in photosynthetic organsims" map. Double-click on that map name and the "Carbon fixation" map opens with E4P highlighted. The Reaction Connections panel shows that there are three associated reactions in the map (designated by **bold**).

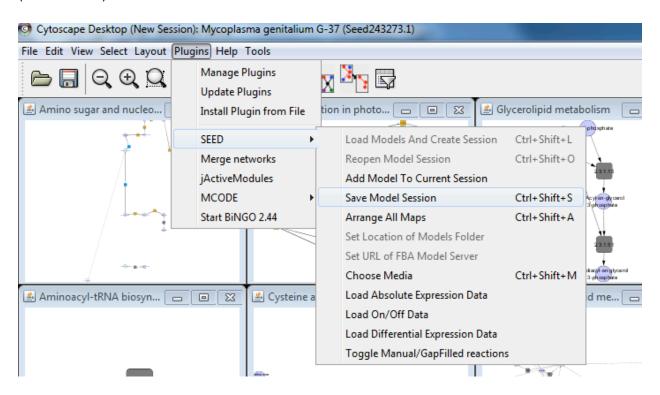


3. Select the reaction labeled with EC 4.1.2.13 in the network display, and right-click and use Move Reaction to move it to the Pentose Phosphate Pathway map. Repeat for the reaction connection between S17P and S7P (EC 2.7.1.11 in the Seed243273.1 Network) and the gap is plugged. Drag the reactions and compounds as needed to clean up the display.

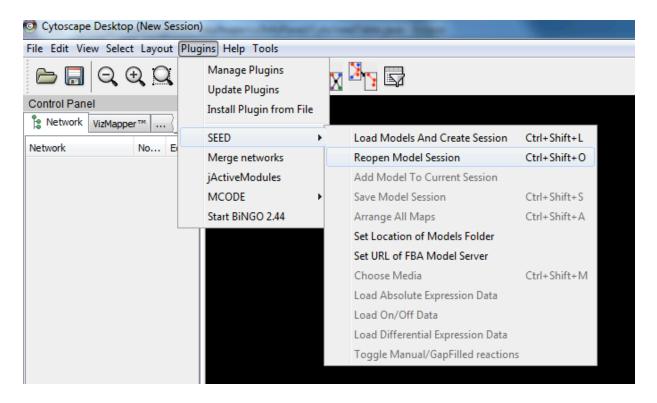


### SAVING AND RESTORING A MODEL SESSION

You can save a model session by selecting *Plugins->SEED->Save Model Session* (Ctrl+Shift+S).

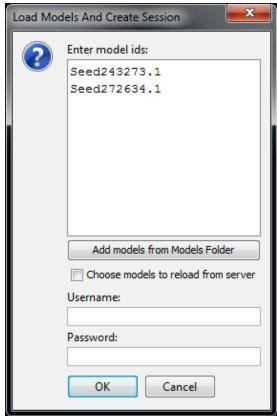


To load a saved model session, first close out of any current Cytoscape session with *Plugins -> SEED -> Close Model Session* (Ctrl+Shift+Q), or start a new instance of the Cytoscape application. Then select *Plugins -> SEED -> Reopen Model Session* (Ctrl+Shift+O). Note: Opening a CytoSEED session file directly from the file system (e.g., by double-clicking on the saved session file) will not correctly restore the session, you must use the *Plugins -> SEED -> Reopen Model Session* menu item.

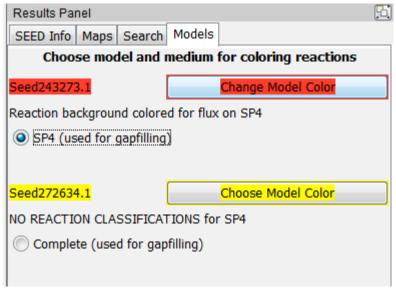


### **COMPARING MULTIPLE MODELS**

Start Cytoscape, select *Plugins -> SEED -> Load Models And Create Session* (Ctrl+Shift+L), and enter multiple model ids into the dialog.



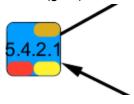
CytoSEED will load both models. Reactions and compounds that are present in more than one model will be represented by the same node in Cytoscape. There will also be an additional tab in the results panel named "Models." Selecting a different model/medium combination in this panel will change the coloring and directionality of reactions based on the reaction classifications for that model in that medium.



Small colored labels on the bottom of reactions and compounds denote the models in which they are present. For example, this reaction is only present in the model associated with red (Seed272634.1).

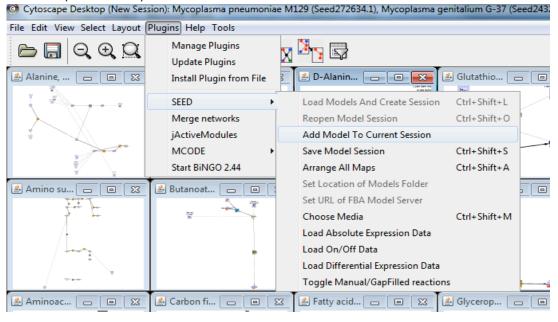


Color labels on the top half of reactions denote whether the reaction has a different classification in a model other than the model currently selected. For example the following reaction is Active (blue) in the currently selected model (Seed272634.1) and medium but is Essential (gold) in the model associated with yellow (Seed243273.1) in the same medium.

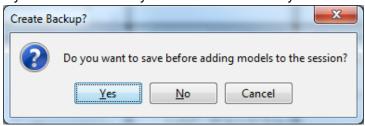


#### ADDING ANOTHER MODEL INTO AN OPEN SESSION

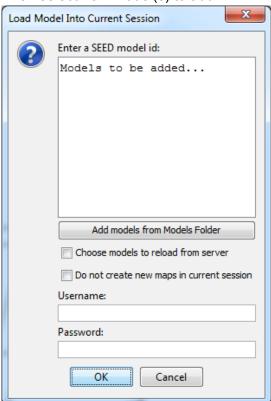
To add another model into a session, click on Plugins -> SEED -> Add Model To Current Session (Ctrl-Shift-A).



CytoSEED will ask if you would like to save your current session before adding another.



Then select new model(s) to add.

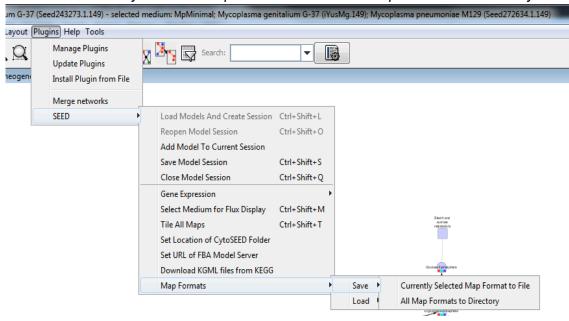


### SAVING AND LOADING MAP FORMATS

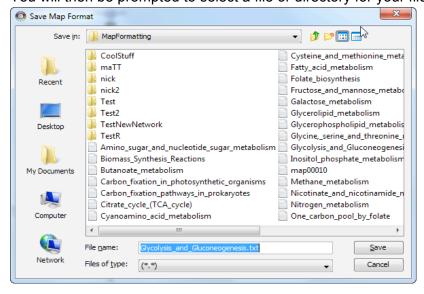
The ability to save map formats allows the user to save the current layout of one or more pathways. These saved formats can be used to quickly apply the layouts to other models. This is different from saving a session because the formats saved are not model-specific and can be applied to any model. If the map is not already present within the current model the system will create it, but only populate it with reactions and compounds actually present in the model.

#### SAVING A MAP FORMAT

To save the format of a map for later use, click on *Plugins -> SEED ->Map Formats -> Save ->* then either *Currently Selected Map Format to File* or *All Map Formats to Directory*.

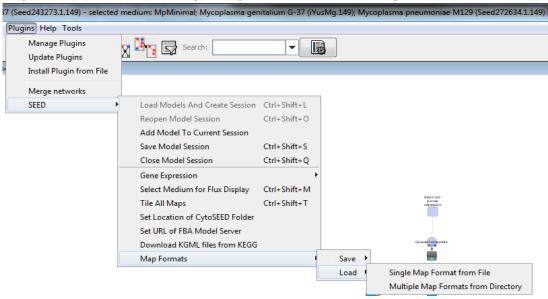


You will then be prompted to select a file or directory for your file(s) to be saved in.

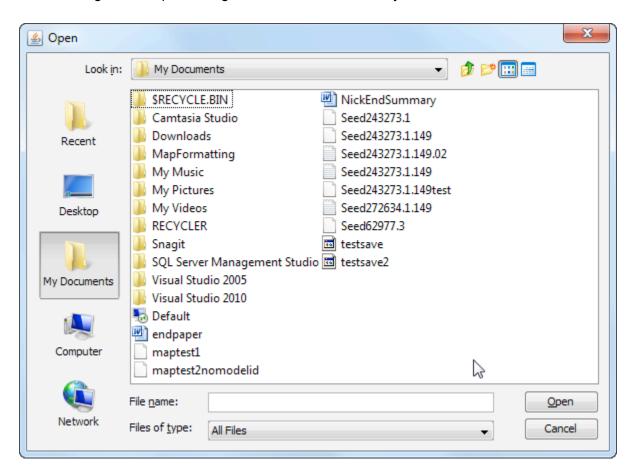


### LOADING A MAP FORMAT

To load saved formats, click on *Plugins -> SEED ->Map Formats -> Load ->* then either *Single Map Format from File* or *Multiple Map Formats from Directory*.



You will be given an open dialog to select a file or directory.



#### OTHER NOTES / KNOWN ISSUES

- CytoSEED is meant for visualization of models and currently does not support editing models. In particular, CytoSEED does not recognize new nodes or edges created in Cytoscape.
- There is currently no undo feature in CytoSEED
- If all reactions in your model are gray and/or purple, you need to have reaction classifications run on your model; contact seed-tech@mcs.anl.gov
- By default, Cytoscape does not show node labels when a large number of nodes are in view in the current network. To override this, go to View -> Show Graphics Details.
- While using CytoSEED, it is recommended that you only use the Layout and Plugin->SEED
  menus in Cytoscape. In particular, do not use the Cytoscape menus to hide or delete
  nodes; use the right-click context menu provided by CytoSEED.
- If a map is maximized when tiling all maps, Cytoscape will automatically maximize the next map selected (Windows) or the previously maximized map (Mac). This appears to be a Cytoscape bug.

## **HOT-KEYS "CHEAT SHEET"**

- Ctrl+Shift+L -- Load model and create session
- Ctrl+Shift+O -- Reopen model session
- Ctrl+Shift+S -- Save model session as
- Ctrl+S -- Save model session
- Ctrl+Shift+M -- Choose media
- Ctrl+H -- Hide selected nodes
- Ctrl+Shift+Z -- Zoom to display entire current network
- Ctrl+Shift+T -- Arrange all maps (Tiled view of all networks)